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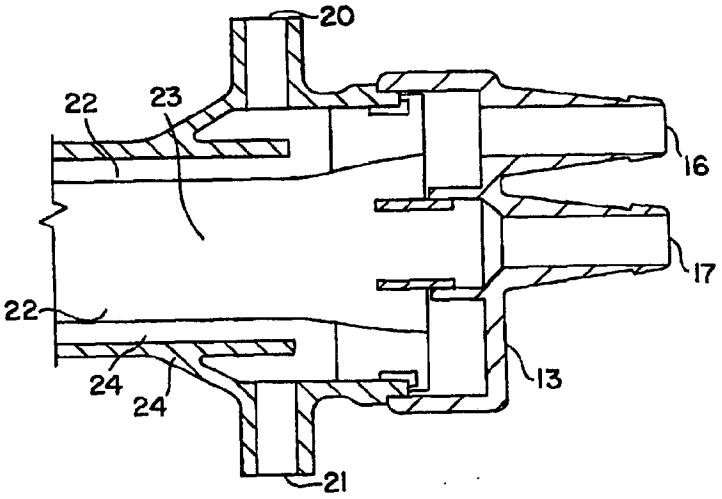

(57) Abstract

A high performance hollow fiber bioreactor having concentric hollow fiber bundles (22, 23) is disclosed. The central hollow fiber bundle (23) supplies culture medium while the outer hollow fiber bundle (22) supplies oxygen needed for cell culture. Methods for use of the high performance bioreactor include, for example, to expand stem cells ex vivo are described.

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HIGH PERFORMANCE CELL CULTURE BIOREACTOR AND METHOD

FIELD OF THE INVENTION

This invention relates to hollow fiber bioreactors. More particularly, this invention relates to a novel high performance bioreactor and to a method for its utilization.

BACKGROUND OF THE INVENTION

Prior art bioreactors conventionally perfuse nutrient media through a single type of hollow fiber. The various disadvantages of such bioreactors include heterogeneous cell mass, difficult procurement of representative cell growth samples, and poor performance due to inefficient oxygenation and an inability to control oxygen levels. Moreover, microenvironmental factors such as pH cannot be effectively controlled. Mixed or co-culture of cells is not possible.

DEFINITIONS

In the specification and claims, the following terms have the meaning set forth below.

HPBr--High Performance Bioreactor.

Extracapillary Space (ECS)--The space within the bioreactor cartridge which is not occupied by the hollow fibers.

Intracapillary Space (ICS)--The totality of the space defined by the lumens of the hollow fibers in a bioreactor.

OXY-1--A hollow fiber oxygenator available from UniSyn Technologies, Inc., 14272 Franklin Avenue, Suite 106, Tustin, California 92680. The OXY-1 bioreactor is provided with 0.2 μm pore size polyethylene hollow fibers woven to form a mat. The active surface area for gas exchange is 1ft^2.

BR110--A small hollow fiber bioreactor commercially available from UniSyn Technologies, Inc. The BR110 hollow fibers are cellulosic, with 10kD molecular weight cutoff (MWc). The active surface area for cell growth is 1.5ft^2.

Micro Mouse BR110--A sterile disposable bioreactor device equipped with a BR110 bioreactor with a length of silicone tubing in the ICS loop for gas exchange available from UniSyn Technologies, Inc.

Cell-Pharm 1000--A hollow fiber bioreactor instrument commercially available from UniSyn Technologies, Inc. The Cell-Pharm 1000 system includes a controller, pH sensor, gas mixture control capability, media reservoir, heater and peristaltic pump and pinch valves.

MAb--Monoclonal antibody.

Mitsubishi Oxygenator Fibers--0.2 μm pore size polypropylene single strands of hollow fibers from Mitsubishi, Japan.

Glucose Utilization Rate (GUR)--The rate at which glucose is consumed from the nutrient media in a batch feed system (in units of mass per unit time, e.g. gm/day) on the day prior to the current sample. This is derived as follows:

$$GUR = F(G_f - G) + V(G_1 - G), \quad G = (G_1 + G_2)/2$$

where G_f is the glucose concentration in IC media feeding (i.e. -4.1g/L); G_1, the glucose concentration (g/L) in IC recirculating media on the day prior to the current sample; G_2, the glucose concentration (g/L) in IC recirculating media in the current sample, F, the IC media feeding rate (L/day), and V, the volume of IC media in reservoir (L).

Lactate Production Rate (LPR)--The rate at which lactate (or rather lactic acid) is produced anaerobically while glucose is consumed. Therefore, one molecule of glucose produces two molecules of

lactic acid without oxygen involved in the reaction. Lactate production rate (LPR), g/day is defined as:

$LPR = [F(L-L_f) + V(L-L_1)] \times 0.09$, $L = (L_1+L_2)/2$

where L_f is the lactate concentration in intracapillary (IC) media (i.e. 0.1 mM); L_1, the lactate concentration (mM) in IC recirculating media on the date prior to the current sample; L_2, the lactate concentration (mM) in IC recirculating media.

SUMMARY OF THE INVENTION

It has been discovered that inefficient oxygenation is a focal constraint upon the efficiency of hollow fiber cell culture devices. The invention provides a novel bioreactor which achieves greatly enhanced nutrient media oxygenation as compared with prior art devices.

Pursuant to the invention, nutrient media is supplied through an inner passage concentric to and hence surrounded by an outer bundle of oxygenator hollow fibers. The inner media passage is provided by a single, media porous tube or by an inner bundle of media supply hollow fibers. The exterior surface of the outer or oxygenator fiber bundle is spaced from the inner wall of the bioreactor housing to facilitate the mixing of cellular material and the sampling or harvesting of product cells.

The high performance bioreactors of the invention is particularly suited for the growth of highly oxygen sensitive cells, such as insect cells used in

the production of biomolecules for commercial purposes.

CONSTRUCTION OF THE HIGH PERFORMANCE BIOREACTOR

This portion of this specification describes, by reference to Figures 1 to 10, the best mode presently known to the inventors for the construction of a bioreactor which embodies the invention.

Description of Figures 1 to 10

Figure 1 is a schematic illustration of a bioreactor of the invention.

Figure 2 is a cross-section of one of two substantially identical ends of a bioreactor as shown by Figure 1.

Figure 3 depicts the hollow fiber housing and the critical components which enable efficient and scaleable fabrication of high performance bioreactor.

Figure 3A is an end view of the hollow fiber housing of Figure 3.

Figure 4 shows the Figure 3 assembly in the mold with potting compound.

Figure 5 illustrates the potted ends of the cartridge after removal from the mold, with the two cut marks for removal of excess adhesive and fiber indicated. Note that the mask component is sacrificial and is removed with excess adhesive. The hollow fiber guide remains imbedded in the adhesive as a permanent part of the end seal in the hollow fiber housing.

Figure 6 depicts the cut end of the bioreactor cartridge.

Figure 7 shows the final assembly including the dual port header, which provides a means to supply media to the inner bundle of fibers and oxygen containing gas to the outer concentric ring of fibers.

Figure 8 is a detailed scale drawing of the fiber guide. This component provides the means for both organizing the two types of fibers, and enables sealing of the central fiber bundle to an independent port from that of the outer bundle of fibers.

Figure 9 is a detailed scale drawing of the sacrificial mask which fits over the portion of the fiber guide to provide a clean surface for sealing the central fiber bundle port.

Figure 10 is a detailed scale drawing of the dual port header for supplying media to the central bundle of fibers and oxygenated gas to the outer fibers independently.

Referring to Figure 1, the bioreactor 10 includes a housing 11 having substantially identical headers 12 and 13 at its input and output ends. Headers 12 and 13 have ports 14, 15, 16 and 17 for the introduction and withdrawal of oxygen containing gas and media.

Figure 2 illustrates an outer oxygenator fiber bundle 22 concentric with an inner media supply fiber bundle 23. The exterior periphery of the oxygenator bundle 22 is separated by space 24 from the inner wall of the housing 11. Header ports 14 and 16 accommodate the introduction of oxygen containing gas into and withdrawal of oxygen containing gas from the oxygenator bundle 22. Header ports 15 and 17 accommodate the passage of nutrient media into and the removal of nutrient media from the nutrient media supply bundle 22.

Product cells or proteins are sampled and harvested from space 24 through ports 18, 19, 20 or 21. Inoculation cells may be introduced through ports 18, 19, 20 or 21.

A preferred method of fabricating a bioreactor embodying the invention includes six major steps now described by reference to Figures 3 to 10.

1. Prepare 4 3/4 inch length fiber bundles from fiber tows with the requisite number of strands. Wrap the end of each bundle with tape so that fibers are held securely.

2. Assemble jacket (10) with the fiber guide (10a) and mask (10b) as shown in Figure 3.

3. Preheat and prespin cellulose fiber bundles for 20 minutes at 1800 RPM and 52°C to remove excess glycerine processing aid. Carefully insert cellulose fibers through the central hole in the fiber guides at each end of the jacket assembly shown in Figure 3A. Similarly, put three bundles of oxygenator fibers through the three lateral holes in the fiber guide and ensure that they are loosely packed. Further drying of the bundles is done at 65°C for 14-18 hours.

4. Place the warm jacket/bundle assembly in a potting mold to encase the free ends of the bundles as shown in Figure 4. This assembly is placed in a centrifuge with premixed polyurethane resin in an applicator. Spin the entire assembly at 1500 RPM for 50-60 minutes at 38-45°C.

The resin enters the jacket assembly (10) through either ports 18 and 20, or 19 and 21.

5. Cut the warm cured resin at the potted ends of the jacket/bundle assembly along the two "cut" lines shown in Figure 5. Remove the cut pieces along with the mask (10b shown in Figure 3), to expose the clean surface of the fiber guide (10a in Figure 3). This surface participates in sealing the header.

6. The header is installed after applying polyurethane resin to the clean surface exposed by removing the mask, and to the outer rim of the jacket (10). Curing occurs at room temperature over a 24 hour period.

Media Supply Fiber Types

Hollow fibers represent a specific group of tubular articles that are within the scope of this invention. Therefore, any porous tube or fiber that is capable of being integrated into a recirculating (ICS) loop, while enabling perfusion of nutrient media to cells growing on the outside, is included in this invention. The following are examples of tubes or fibers preferred for use in this invention:

(i) Hollow fibers and tubes with dialysis, ultrafiltration and microfiltration properties, i.e., (a) molecular weight cutoff (MWc) ranging from 1 kD to 1,000 kD; and (b) pore size ranging from 0.01 μm to 5.0 μm,

The most preferred range is from 10 kD MWc and about up to 1 μm pore size.

(ii) Materials of construction include polymers, graphite, ceramics (including porous glass fiber) and metals (e.g., stainless steel). Typically, polymers are required to have good physical properties (e.g., tensile strength, melt temperature and glass transition temperature). These include cellulose, polyethylene, polypropylene, polysulfone (and other engineering thermoplastics), polymethyl methacrylate, polyacrylonitrile, various polymer blends and the like.

Specifically preferred media supply fibers are from cellulose polymers, have a MWc of 10-1,000 kD and a pore size of 0.01-1.0 μm.

Oxygenator Fiber Types

Hollow fibers are the only tubular configuration selected for oxygenation in this invention. These hollow fibers can be porous or non-porous. Porous hollow fibers for oxygenation are typically hydrophobic (like those made from polyethylene, polypropylene, polytetrafluoroethylene (Teflon) and the like). Examples of non-porous hollow fibers include (but are not restricted to) silicone, and silicone copolymer capillary tubing. They typically have high gas permeability through the solid wall structure.

Porous hydrophobic hollow fibers are most effective for gas exchange when the pore size is in the range of 0.01 to 0.5 μm. Optimally, pore size should be in the range of 0.1 to 0.2 μm.

Specifically preferred oxygenator fibers are formed from polypropylene and a pore size of 0.15 μm.

Discussion of Operation

The HPBr is equipped with the means for recirculating nutrient media via the lumen (or ICS) of a hollow fiber bundle or other tubular structures centrally located in the bioreactor jacket. These hollow fibers or tubes are selected for their permeability properties, and in some instances for their surface properties (e.g., hydrophilicity or hydrophobicity). Media profused through the porous wall of these structures bath cells that have been inoculated in the ECS.

O_2/CO_2 gas mixtures are supplied to the HPBr through the lumen tube or a bundle of hollow fibers that are situated concentric to the centrally placed hollow fibers or tubes. The gas mix flows counter current to the flow of media. One important operating parameter is the gas pressure in the

oxygenator fibers. The optimal pressure is a function of the number of strands of fibers used, and the pressure of the perfusing media in the ECS. For example, a bioreactor embodiment described in the examples that had 180 Mitsubishi polypropylene fibers showed a bubble point of >2.5-3 psi. For a HPBr with 540 oxygenator fibers, the bubble point was closer to about 2 psi. As cell mass increases and cells adhere directly to the fibers, the bubble point will tend to increase.

Table 1 illustrates the relationship between the number of oxygenator and media fibers for two types of HPBr's described in Example II (i.e., Type I and II). Two additional HPBr configurations (Type III and IV), and an OXY-1/BR110 combination are included for comparison. The figure demonstrates that as the ratio of oxygenator fiber surface area to media fiber surface area increases, oxygenation efficiency also increases at the expense of the extracapillary space available for cell culture.

HPBr Type IV represents a more extreme case covered by this invention, where the annular cell culture space is within the range for a BR110 (conventional) bioreactor. The ECS of 11.8 mL is within the range expected from about 70% (maximum) fiber packing density for a BR110 or OXY-1. (The maximum space in the potted jacket with absolutely no fiber present is about 22 mL.) For the embodiment described in Example II, the oxygenator/media fiber surface area ratio range is between 0.03 to 5.5. The preferred range is between 0.7 and 2.6. As this data shows, the invention may be regarded as an oxygenator device within which cells are cultured, and media supply is provided by a relatively few porous hollow fibers or a large porous tube, e.g., a graphite tube.

TABLE 1

Relationship Between Oxygenator and Media Supply Fibers in HPBr

	OXY-1/ BR110 Combination	High Performance Bioreactors Type I	Type II	Type III	Type IV*
Oxygenator Fiber S.A. (ft^2)	1.0	0.1	0.3	0.5	1.1
Media Fiber S.A. (ft^2)	1.5	0.2	0.2	0.2	1.2
Oxy/Media Fiber Ratio	0.7	0.5	1.5	2.5	5.5
Extracapillary Space (mL)	n/a	19.5	18.0	16.4	11.8

*Note: Based on 2000 media fibers at a maximum packing packing density (i.e., ~ 70%).

The gas composition is important in defining the efficiency of oxygenation as well as the pH in the micro-environment for cell growth created in the ECS. Illustrative data showing the effect on pH is presented in Example II. This invention permits more accurate monitoring and control of pH in the optimal range required than had been possible previously.

Provision is made in the bioreactor for an annular space between the outer most oxygenator fibers and the inner wall of the jacket. This space is a specially designed part of the ECS for containing cell mass. This culture space facilitates mixing when the bioreactor is (optimally) periodically rotated to-and-fro (by 120 degrees). Further, it facilitates sampling and harvesting of products (particularly intact viable cells, e.g., for cellular therapy applications). This space also enables microcarriers for anchorage dependent cells to be contained in the HPBr. A specific instance

where this is critical is in the culture of hematopoietic stem cells for cellular therapy applications. Stem cells are grown better in the presence of anchorage dependent stromal cells (including endothelial cells). However, stem cells are anchorage independent. Therefore, stromal cells immobilized in or on microcarriers can be co-cultured with stem cells. The micro-environment thus created will facilitate the hitherto unrealized goal of long term ex-vivo expansion of stem cells. High yields and viability for cells from this cell culture space are achievable.

Finally, expensive growth factors and cytokines that are vital to the growth and differentiation of various cell types can be applied in relatively small quantities to the ECS to further tailor the micro-environment.

Important and unique features of the invention, both separately and in combination, include: a relatively high ratio of gas to media supply fibers in a single jacket; control of oxygenation and pH by gas composition, pressure and flow rate; relatively low fiber packing density to provide an extracapillary space for growing large cell mass and containing microcarriers; porous tubes or hollow fibers are employed to supply media and porous or non-porous hollow fibers are concentrically arranged to supply oxygenated gases.

Two different types of hollow fibers are used, that are made from different materials. This is represented by the examples in this invention.

One type of fiber (e.g., microporous polypropylene), is utilized where media perfusion and gas exchange in the respective bundles are achieved and controlled by regulating pressures. In

applications where anchorage independent cells are being cultured at very high densities, hydrophobic fiber may be preferred so that cells grow primarily in suspension and not on the fibers.

Media supply may be provided by a single porous tube or by a bundle of hollow fibers.

Coatings are applied to hollow fiber or tube surfaces to influence cell attachment (either positively or negatively). For example, cellular matrix molecules found in connective tissue between cells in-vivo coated onto bioreactor fibers facilitates adhesion of anchorage dependent cells like endothelial cells and Chinese hampster (CHO) cells. Other molecules containing the active amino acid sequence arginine-glycine-aspartic acid, called "RGD" found in matrix molecules also provide this benefit. Cellular matrix molecules coated onto HPBr surfaces facilitate a microenvironment conducive to efficient gene transfer into hematopoietic stem cells.

Additional surface area for anchorage dependent cells is achieved by introducing microcarriers to the culture space in the ECS. Co-culturing of different cell types to gain synergistic benefit is permitted (e.g., stem cells and stroma).

Addition of oxygen carriers (particularly hemoglobin and perflourochemical emulsions) to the ECS (or ICS), greatly enhances the ability to accurately control oxygen delivery to the cells and the rapid growth of cell mass.

EXAMPLE I

Two 15 day cell culture experiments were conducted with 3C11 hybridoma cell line, that secrets IgG_1 monoclonal antibody in a UniSyn Cell Pharm 1000 pilot scale bioreactor system. In run 1, three UniSyn BR110 bioreactor cartridges (1.5 ft^2 cellulose

hollow fibers) were equipped with a UniSyn OXY-1 oxygenator (1 ft^2, 0.2 μm pore size polyethylene oxygenator fibers) for oxygen mass transfer. In run 2, three high performance bioreactor (HPBr) equivalently sized cartridges (#1, #2, and #3) were fitted to evaluate the capacities of HPBr in 3C11 cell culture. These HPBr's all had 450 cellulose hollow fibers (with 10 kD M.W. cut-off) but #1 and #2 had different amounts of oxygenation fibers (in the form of a mat, as used in OXY-1); and, #3, 504 polyethylene oxygenation fibers.

Figure 11 is a schematic of the flow path for three BR110 cartridges with one OXY-1 cartridge used for run 1. Media recirculation rate was established by setting the RPM of the media recirculation peristaltic pump. The mixed gas containing CO_2 and air was pumped through the oxygenator counter current to media recirculating in the ICS loop of the bioreactors by using the gas peristaltic pump. Calibrating pH and temperature probes were performed according to the operator's manual. To control the pH in the range of 7.0 to 7.4 in ICS media, the control unit of the Cell Pharm 1000 adjusted the CO_2 percentage in the mixed gas automatically. Also, this control unit maintained the constant temperature at 37°C. Figure 12 is a schematic of the flow path for the three HPBr cartridges in run 2. No change has been made for ICS media flow path relative to run 2. However, a modified gas flow path was designed. This involved connecting the gas mixture supply to both the three HPBr's and the media reservoir. It should be noted that this crude modification of the existing Cell Pharm 1000 did not provide optimal control of gas flow and back pressure. Connecting the gas supply with the ICS

recirculation media reservoir facilitated pH control by the Cell Pharm 1000 system controller.

The entire flow path including all tubing, filters, probes, and reservoirs, renew, and waste bottles were autoclaved at 120°C for thirty minutes on the liquid cycle. Final assembly included mounting the bioreactor cartridge into the Cell Pharm 1000 System. Contamination check and system flush were set to run for 24 hours to ensure sterility, removal of glycerine (processing aid for cellulose fibers), and allow for system stability.

Media conditions:

(i) ICS--5% fetal bovine serum (FBS) + 1% penicillin/streptomycin + 2% glutamine in 1000 mL DMEM.

(ii) ECS--20% FBS + 1% penicillin/streptomycin + 2% glutamine in 1000 mL DMEM.

Each bioreactor cartridge, including the BR110 and HPBr, were inoculated with the same 3×10^8 cells (over 90% viability) into the ECS by using two sterile, 10 mL syringes with attached 18 gauge needles. One syringe contained 5 mL of ECS media and the cells. The second syringe was empty and used to collect the media displaced during the inoculation.

Product harvest was accomplished in a manner similar to the inoculation procedure. Harvesting was typically done three times a week and 10 mL/each, and the standard operating procedure for this Cell Pharm 1000 System was followed for maintaining cell culture daily.

Process parameters in the cell culture systems were set up to monitor glucose uptake, lactate production, NH_3 production, MAb production and perfusion rate of ICS media. Since one cannot

directly estimate the cell mass present in the hollow fiber bioreactor at any given time, the MAb production may be regarded as a means for measuring the effect of oxygen mass transfer within the two types of bioreactors in run 1 and 2. MAb concentration in the harvest was analyzed by using radial immunodiffusion assay, glucose, lactate, and NH_3 sampled from the ICS of the bioreactor were analyzed by using a Kodak Ektachem Analyzer. The perfusion rate was determined by measuring the media level of renew bottles. Initial perfusion rate was started at 240 mL/day and was increased step by step and maximized up to 1000 ml/day until the end of the runs according to either the glucose concentration (when it fell as low as 1.5 g/L) or lactate concentration (when it reached as high as 20 mM).

Cumulative MAb production in each HPBr cartridge (run 2) for 3C11 cells, are compared to that in each BR110 (run 1) in Figure 13. The ratio of lactate production rate to glucose consumption rate was developed as an indicator to evaluate the effect of oxygen mass transfer. This ratio was significantly lower in Run 2 (shown in Figure 4) compared to Run 1. These results suggest that the oxygenator fibers in HPBr cartridges help to reduce the resistance of oxygen mass transfer to the grow cells. This was especially true for the HPBr cartridge with the Mitsubishi oxygenator fibers that gave higher MAb production levels than those in the other two HPBr's which have polyethylene oxygenator fibers. It was assumed that the relatively high dissolved oxygen concentration in the ECS lets cells grow more efficiently.

EXAMPLE II

A 25 day cell culture experiment (run 1) was conducted with 3C11 hybridoma cell line, that secrets IgG_1 monoclonal antibody. A test system was designed to evaluate the performance of three HPBr cartridges under controlled conditions. As a reference, a UniSyn Micro Mouse BR110 small scale bioreactor with a UniSyn OXY-1 oxygenator was employed in run 2. Run 3 consisted of a UniSyn Micro Mouse BR110 small scale bioreactor with a silicone tubing for oxygen mass transfer by diffusion.

Run 1 used the test system that was designed to operate HPBr's with the media flow path and the gas flow rate and pressure manually controlled (see Figure 14). A peristaltic pump assembly was used with three pump heads to provide the same media recirculation flow through each HPBr at a flow rate of 100 mL/min. Temperature in the ECS of each HPBr was controlled at 37°C by immersing the three media reservoirs in a thermostatically controlled recirculating water bath. The ICS media pH was controlled in the range from 7.00 - 7.30 by aerating the space above the media in the reservoir with CO_2 in 75% to 85% air. This gas mix was also used to supply gas to the three HPBr cartridges with a constant flow rate of 38 mL (std(/min and back pressure of 1.5 psi.

The three HPBr cartridges each had 450 cellulose hollow fibers for supplying media to the cells by perfusion. Both HPBr #1 and #2 had 180 Mitsubishi oxygenator fibers. The number of Mitsubishi oxygenator fibers in HPBr #3 was three times higher than that in #1 and #2. For run 1, the contamination check and system flush were preformed according to

the instructions found in the Micro Mouse operating manual.

Run 2 was assembled in an oven for maintaining a constant temperature (37°). Premixed air and CO_2 was passed through the OXY-1 oxygenator at a rate of about 70 mL(std)/min counter current to the direction of the loop recirculating media in the ICS of the BR110 bioreactor. To control pH within the range from 7.0 to 7.4, the ratio of CO_2 to air was adjusted by using valves on two gas flow meters.

Run 3 was mounted in a CO_2 incubator for maintaining a constant temperature (37°C) and constant pH (from 7.0 to 7.2) in the IC media was established by setting the ratio of 7% CO_2 to 93% air in incubator.

Media conditions:

(i) ICS--5% FBS + 1% penicillin/ streptomycin + 2% glutamine in 1000 mL DMEM.

(ii) ECS--20% FBS + 1% penicillin/ streptomycin + 2% glutamine in 1000 mL DMEM.

3×10^8 cells (91% viability) were inoculated in the ECS by using two sterile, 10 mL syringes with 18 gauge needles. One syringe contained 5 mL of ES media and cells. The second syringe was empty and used to collect the media displaced during the inoculation. Both run 2 and 3 were inoculated with 4×10^8 cells (95% viability) in the ECS using the method illustrated above. The standard operating procedure for run 2 and 3 was followed for maintaining cell culture daily. For run 1, daily adjustment of back pressure and gas flow rate, sampling from each ICS media and harvesting from ECS of each cartridge were carried out.

Process parameters in the cell culture systems were set up to monitor glucose uptake, lactate and NH_3 production and MAb production. Cumulative MAb production was monitored to determine the effect of different oxygen mass transfer methods exemplified in runs 1-3.

ICS media for the bioreactors was replaced with fresh media when either the glucose concentration fell below 2.0 g/L or lactate concentration was higher than 20 mM. For run 2 and run 3, harvesting of MAb from the ECS of the bioreactor was done three times weekly and a volume of 10 mL/each (as defined by the Micro Mouse protocol). MAb production was analyzed by using radial immunodiffusion assay, and glucose, lactate, NH_3 concentration in harvesting of the ECS of HPBr's were also analyzed. The difference between the levels for these cell metabolites found in the ICS and the ECS of HPBr were noted.

Figure 15 shows the cumulative IgG_1 produced in each HPBr cartridge and in runs 2 and 3. HPBr #3 appeared to be superior in MAb production compared with HPBr #1 and #2. A similar conclusion was drawn when the HPBr was also compared with BR110 bioreactor in run 2 and run 3. This suggests that the increase of oxygenator fibers in HPBr can significantly enhance cell growth and MAb production due to the increased oxygen supply. Also the ratio of LPR to GUR was calculated for HPBr #3 and found to be significantly lower than other bioreactors. This signifies more efficient utilization of glucose in the presence of increased oxygen in the ECS.

Figure 16 compares the lactate/glucose ratio for various bioreactors. The lowest ratio was shown by HPBr devices, and these low values remain below that of the conventional bioreactors and oxygenation

methods for prolonged cell culture runs. Ideally, the ratio should be as close to zero as possible throughout the cell culture run, signifying optimal metabolic utilization of glucose.

Table 2 is a comparison of bioreactor and oxygenator properties. It also contains the relative antibody productivity of bioreactor/oxygenator combination as a function of their physical properties. In this embodiment, as the number (or surface area) of oxygenator fibers increase, and the ratio of oxygenator to media fiber surface areas increase, bioreactor productivity also increases.

TABLE 2

Comparative Bioreactor and Oxygenator Properties

Property	BR110 (Bioreactor)	OXY-1 (Oxygenator)	HPBr Type 1 Media	HPBr Type 1 Oxygenator	HPBr Type II Media	HPBr Type II Oxygenator	HPBr Type III Media	HPBr Type III Oxygenator
Material	Cellulose	Polyethylene	Cellulose	Polypropylene	Cellulose	Polypropylene	Cellulose	Polypropylene
MWC or Pore Size	10 kD	0.2 um	10 kD	0.2 um	10 kD	0.2 um	10 kD	0.2 um
Surface Area (O.D.) (ft^2)	1.5	1.0	0.2	0.1	0.2	0.3	0.2	0.5
Number of Fibers	3150	1684	450	180	450	540	450	900*
Permeability of Media Fibers (mL/hr. mmHg)	0.2	n/a	2.8×10^{-2}	n/a	2.8×10^{-2}	n/a	2.8×10^{-2}	n/a
Typical IgG_1 Productivity (mg) for 3C11 Hybridoma after 14 days of culture (batch run)	23		32,22		68		Data not available at this time.	

*Note: Maximum number of oxygenator fibers possible in this embodiment of HPBr is about 2000 (i.e., 1.1 ft^2). This assumes a packing density of 70%, which is in the range that is typical of conventional hollow fiber devices in general.

Figure 17 illustrates the level of pH control achievable with the HPBr. The particular data depicted is for HPBr #3. pH control regulates biomass production vs. MAb production. Lower pH, e.g., 7.0, tends to encourage cells to remain in the exponential growth phase to produce high biomass. Higher pH, e.g., 7.4, tends to encourage cells to remain in the steady state phase and therefore exhibit higher productivity.

CLAIMS:

1. A hollow fiber bioreactor comprising

a housing

said housing having an input end, an output end, a first port adjacent said input end and a second port adjacent said output end;

a first, interior bundle of porous hollow fibers for supplying nutrient media within said housing;

a second, exterior bundle of hollow fibers for providing oxygen containing gas within said housing;

said first and second bundles of hollow fibers being concentric;

the exterior surface of said second bundle of hollow fibers being spaced from the interior wall of said housing;

an input header for the input end of said housing

said input header having a first port for the introduction of nutrient media into said first, interior bundle of hollow fibers and a separate second port for the introduction of oxygen into said second exterior bundle of hollow fibers; and

an output header for the output end of said housing;

said output header having a first port for the removal of media from said first interior bundle of hollow fibers and a second port for the removal of oxygen containing gas from said second exterior bundle of hollow fibers.

2. A hollow fiber bioreactor as defined by claim 1 in which said first, interior bundle of porous hollow fibers have a molecular weight cut-off of from 1 kD to 1,000 kD and a pore size of from 0.01 μm to 5.0 μm.

3. A hollow fiber bioreactor as defined by claim 1 or claim 2 in which said first, interior bundle of hollow fibers comprises cellulose, polyethylene, polypropylene, polysulfone, polymethyl methacrylate or polyacrylonitrile fibers.

4. A hollow fiber bioreactor as defined by claim 1 in which said second exterior bundle of hollow fibers are porous hydrophobic fibers comprising polyethylene, polypropylene or polytetrafluoroethylene having a pore size of from 0.01 to 0.5 μm.

5. A hollow fiber bioreactor as defined by claim 1 in which said second exterior bundle of hollow fibers comprises non-porous, gas permeable silicone or silicone copolymer fibers.

6. A hollow fiber bioreactor as defined by claim 1 in which the ratio of the surface area of said second exterior bundle of hollow fibers to the surface area of said first interior bundle of hollow fibers is from 0.03 to 5.5.

7. A hollow fiber bioreactor as defined by claim 6 wherein cellular matrix or synthetic molecules containing amino acid sequence arginine-glycine-aspartic acid coat the available surfaces within the housing.

8. A hollow fiber bioreactor as defined by claim 6 in which a combination of carbon dioxide and an oxygenated gas is used to control pH and oxygen levels in the extracapillary space.

9. A hollow fiber bioreactor as defined by claim 6 including oxygen carriers in the extracapillary space or in the intracapillary space.

10. A hollow fiber bioreactor as defined by claim 6 in which hemoglobin or perfluoro chemical emulsions are present in the extracapillary space or in the intracapillary space.

11. A hollow fiber bioreactor as defined by claim 6 for the culture of insect cells.

12. A hollow fiber bioreactor as defined by claim 1 further comprising microcarriers for anchorage dependent cells in said space between the exterior surface of said second bundle of hollow fibers and the exterior wall of said housing.

13. A hollow fiber bioreactor as defined by claim 12 in which stromal cells are immobilized in or on said microcarriers for co-culture with hematopoietic stem cells.

14. A bioreactor comprising a housing

an annular bundle of gas permeable hollow fibers within said housing;

a lumen passing through said annular bundle of hollow fibers;

means for passing an oxygen containing gas through said hollow fibers of said annular bundle;

means for passing nutrient media through said lumen passing through said annular bundle of hollow fibers.

15. A bioreactor as defined by claim 14 further comprising a porous tube positioned within said lumen passing through said annular bundle of hollow fibers.

16. A bioreactor as defined by claim 14 further comprising a bundle of porous hollow fibers positioned within said lumen to supply nutrient media within said housing.

17. A method for the ex vivo expansion of stem cells which comprises

providing a hollow fiber bioreactor having extracapillary space

said extracapillary space containing microcarriers having stromal cells immobilized thereon, and

concurrently culturing stem cells and said stromal cells in said bioreactor.

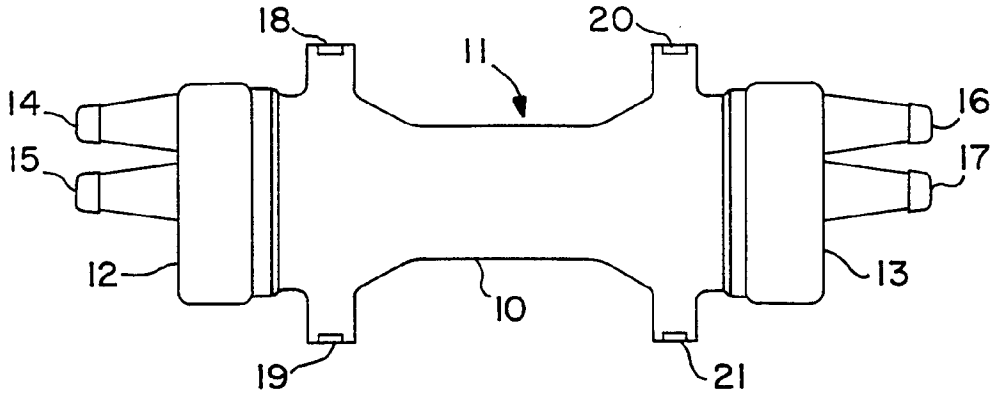

FIG. 1

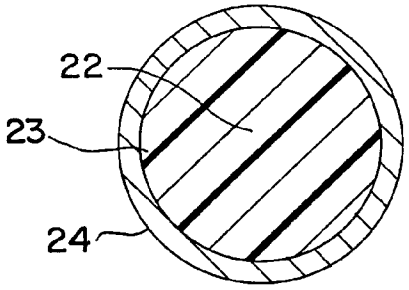

FIG. 2A

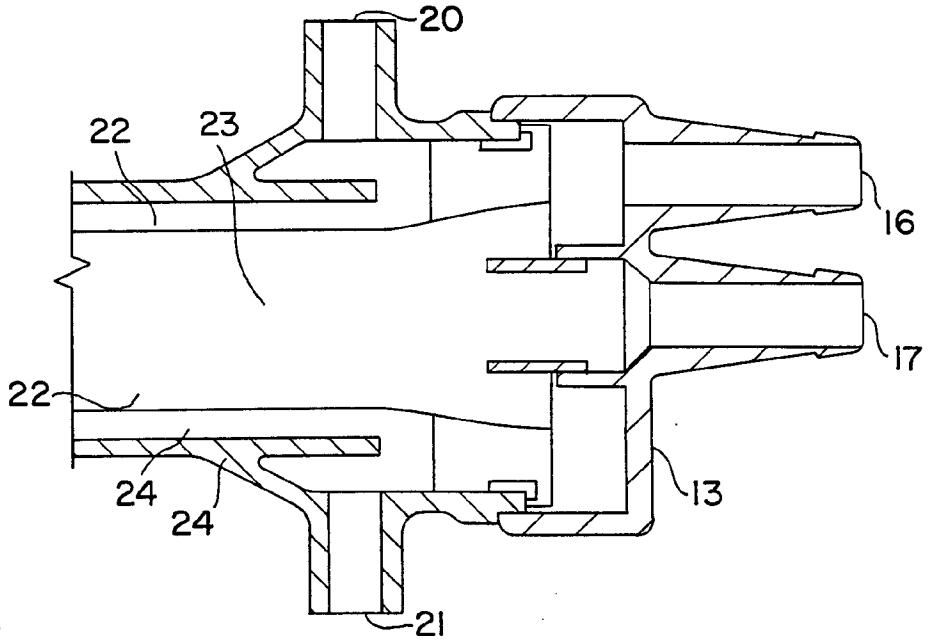

FIG. 2

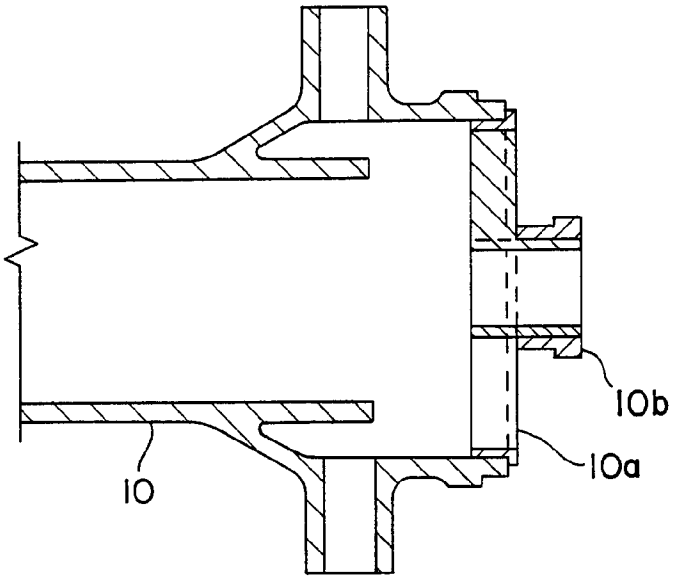

FIG. 3

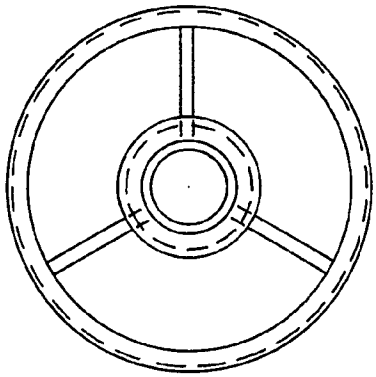

FIG. 3A

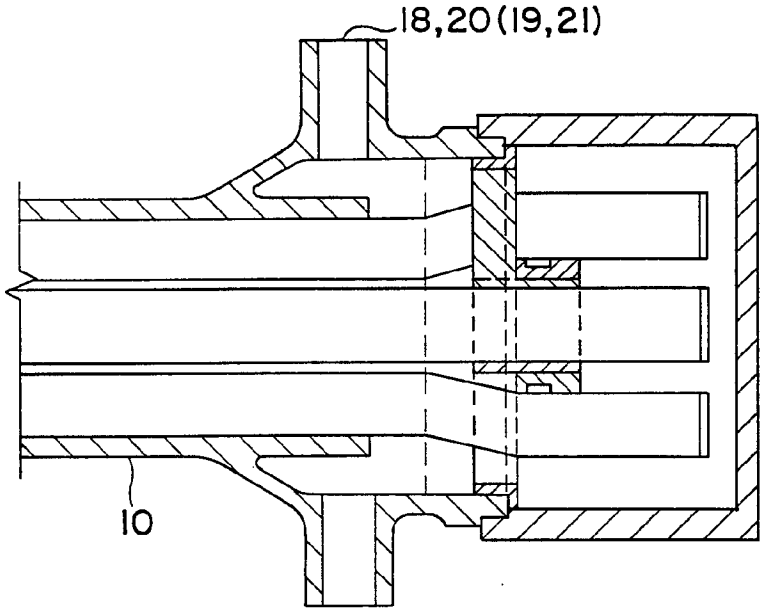

FIG. 4

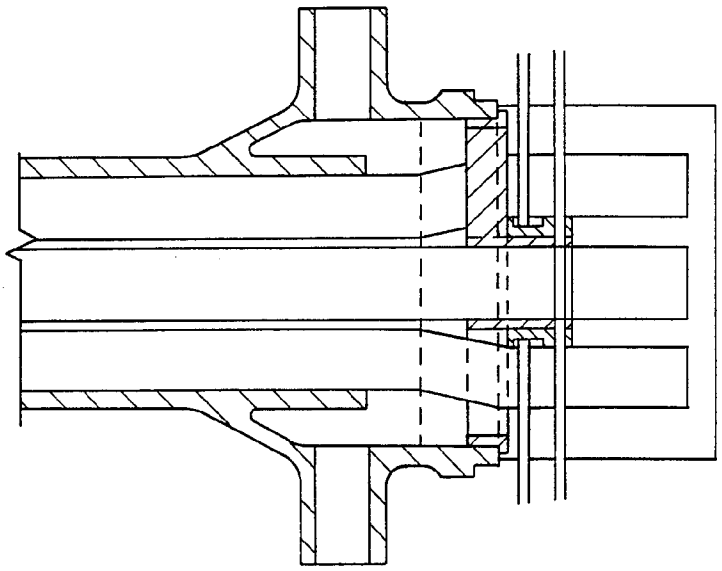

FIG. 5

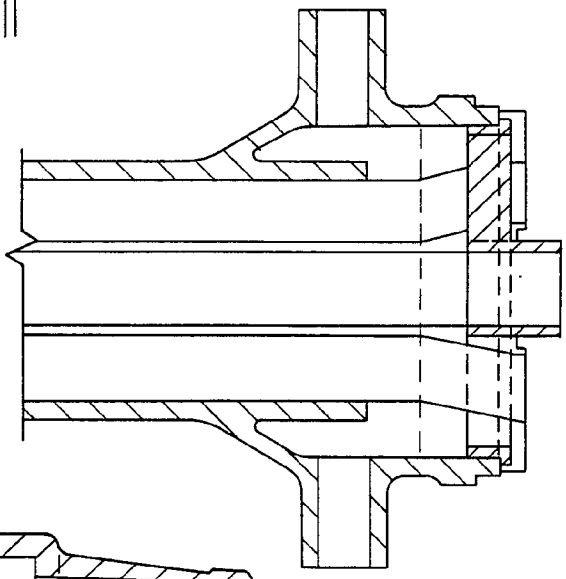

FIG. 6

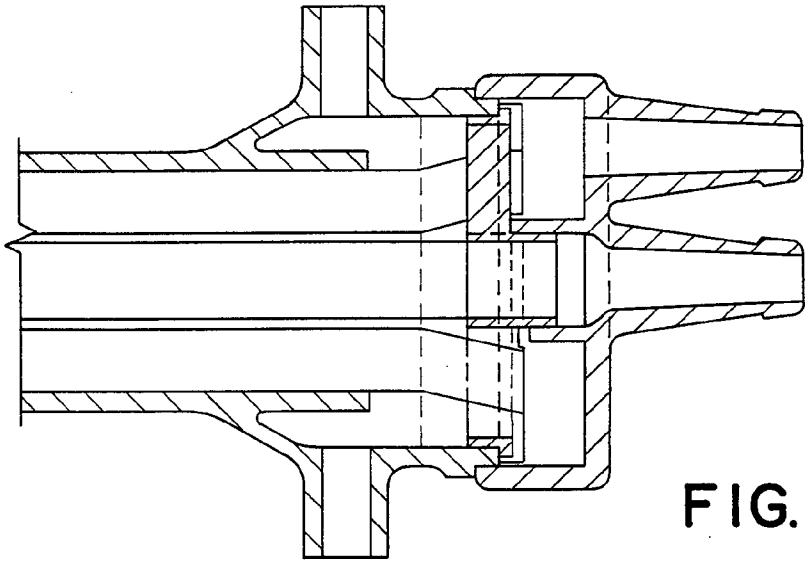

FIG. 7

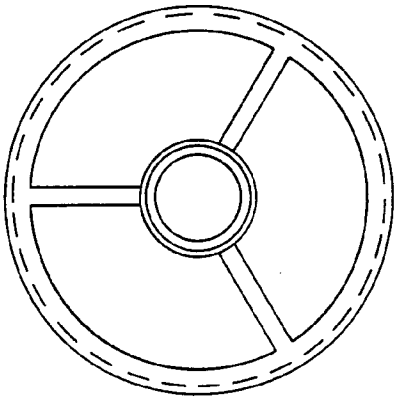

FIG. 8

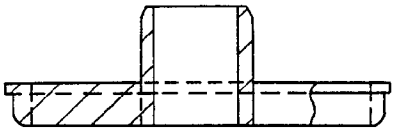

FIG. 8A

FIG. 9

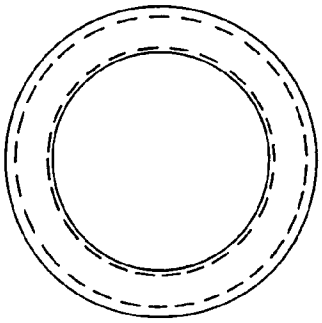

FIG. 9A

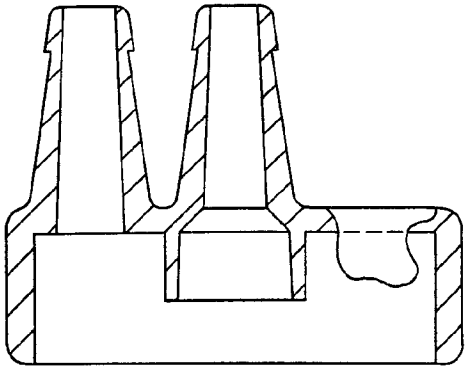

FIG. 10

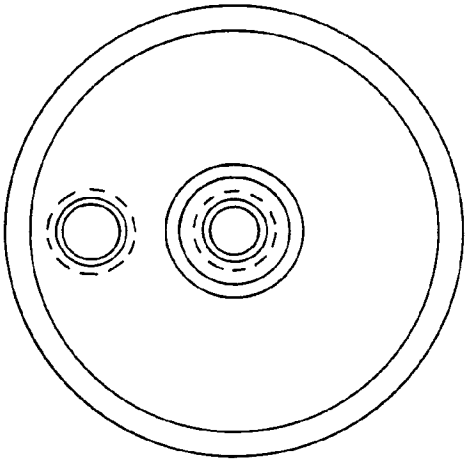

FIG. 10A

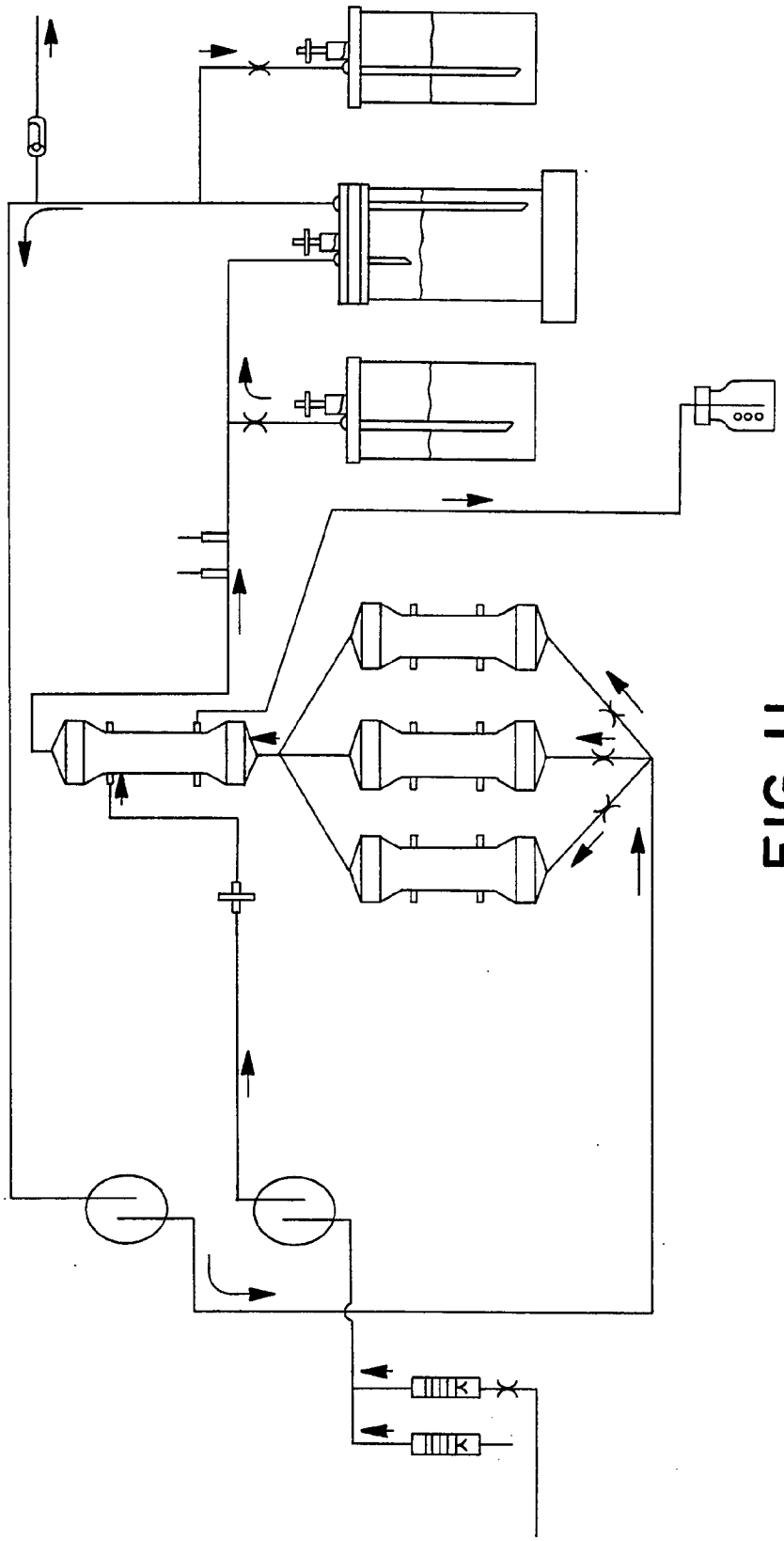

FIG. 11

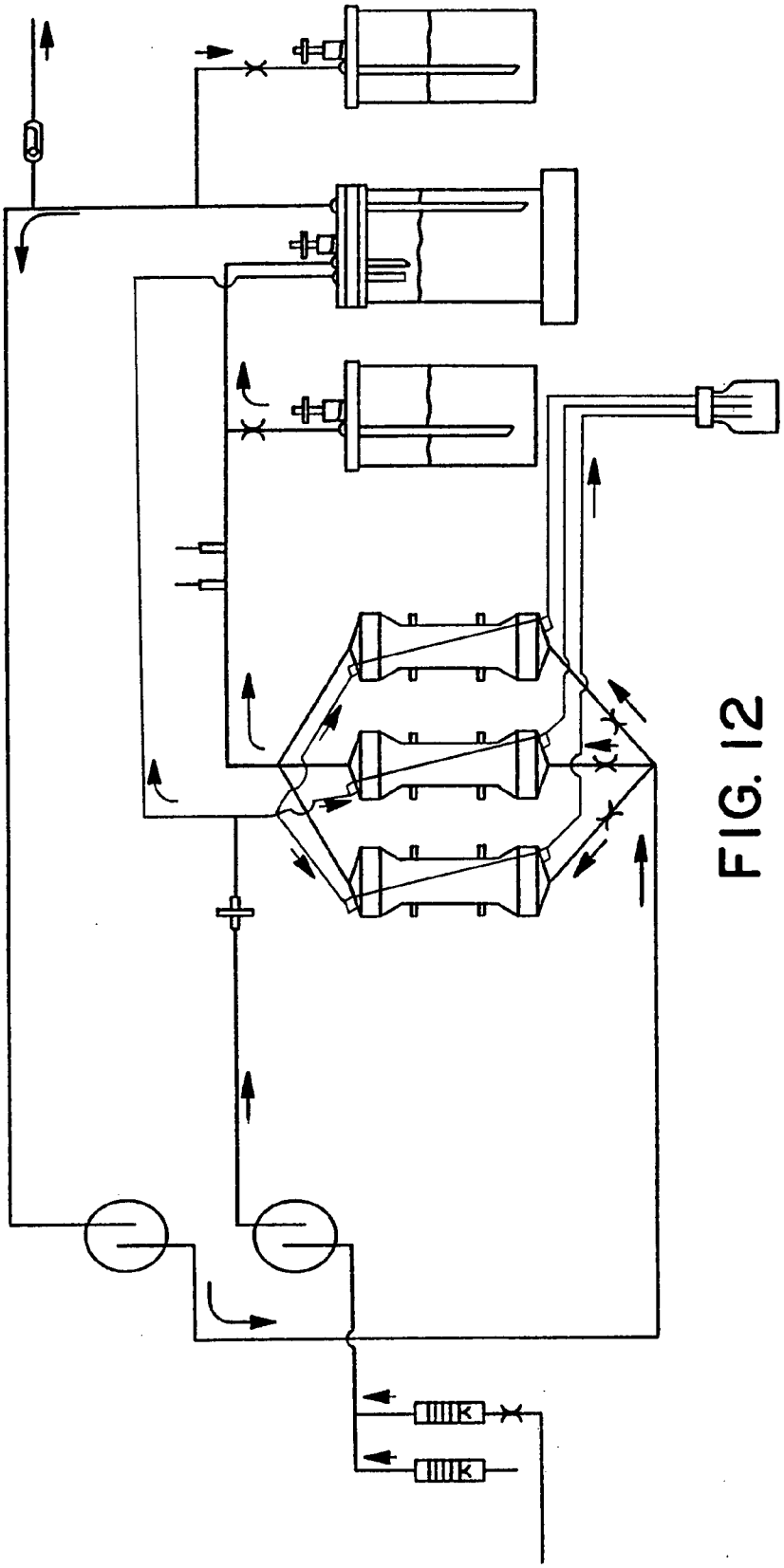

FIG. 12

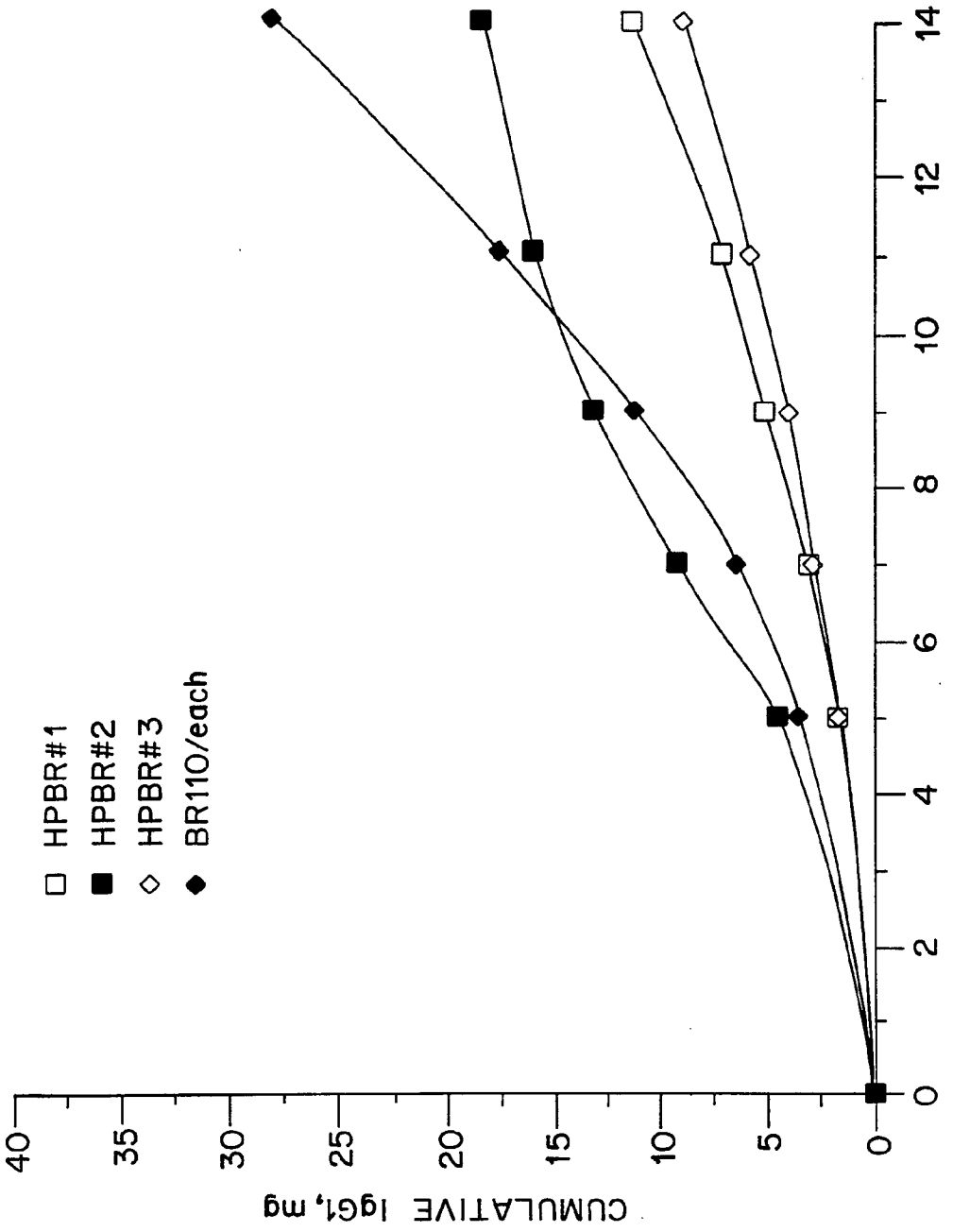
CUMULATIVE IgG1, mg
0
5
10
15
20
25
30
35
40
DAY
0
2
4
6
8
10
12
14
HPBR#1
HPBR#2
HPBR#3
BR110/each

FIG. 13

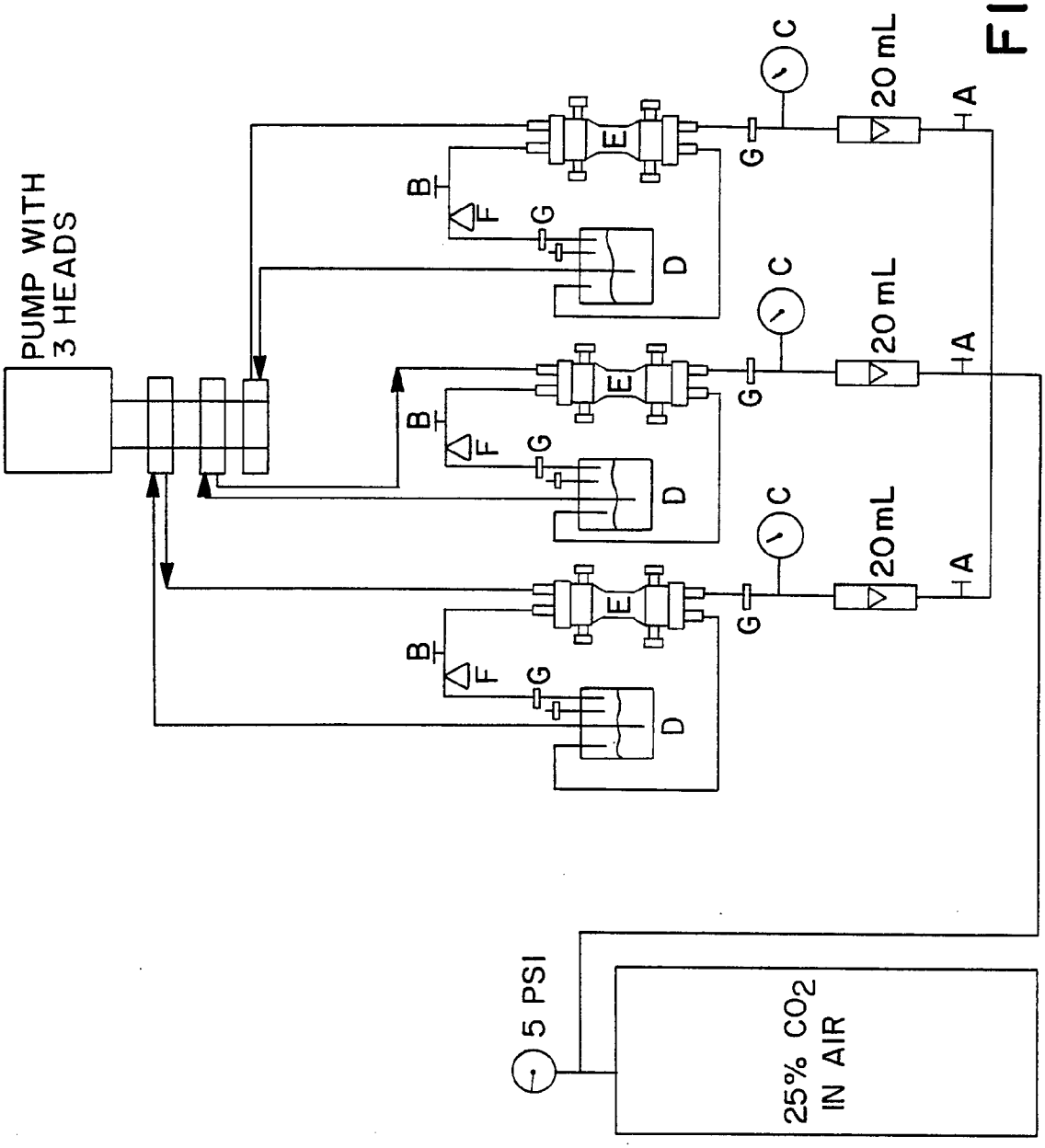

FIG. 14

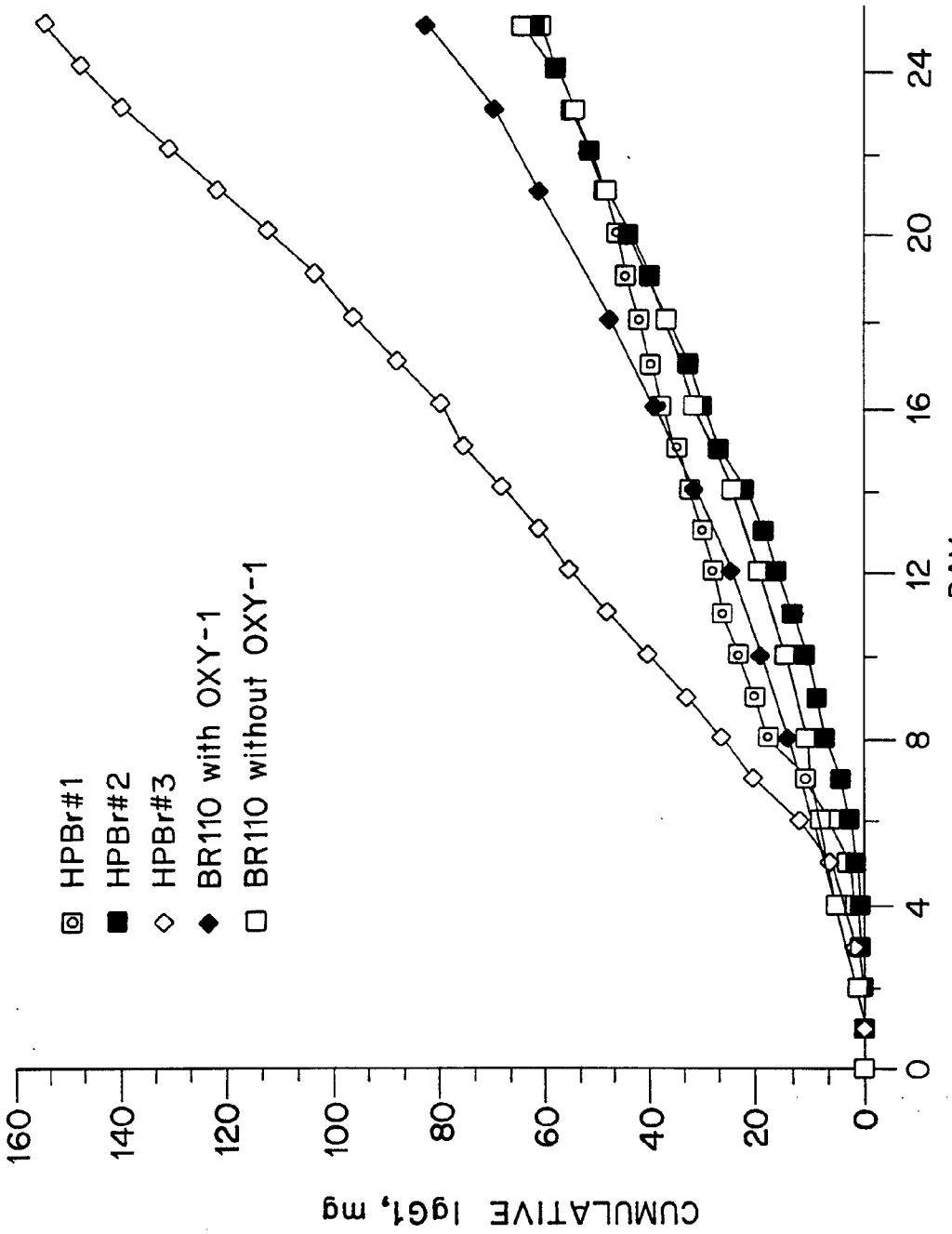
CUMULATIVE IgG1, mg
0
20
40
60
80
100
120
140
160
0
4
8
12
16
20
24
DAY
HPBr#1
HPBr#2
HPBr#3
BR110 with OXY-1
BR110 without OXY-1

FIG. 15

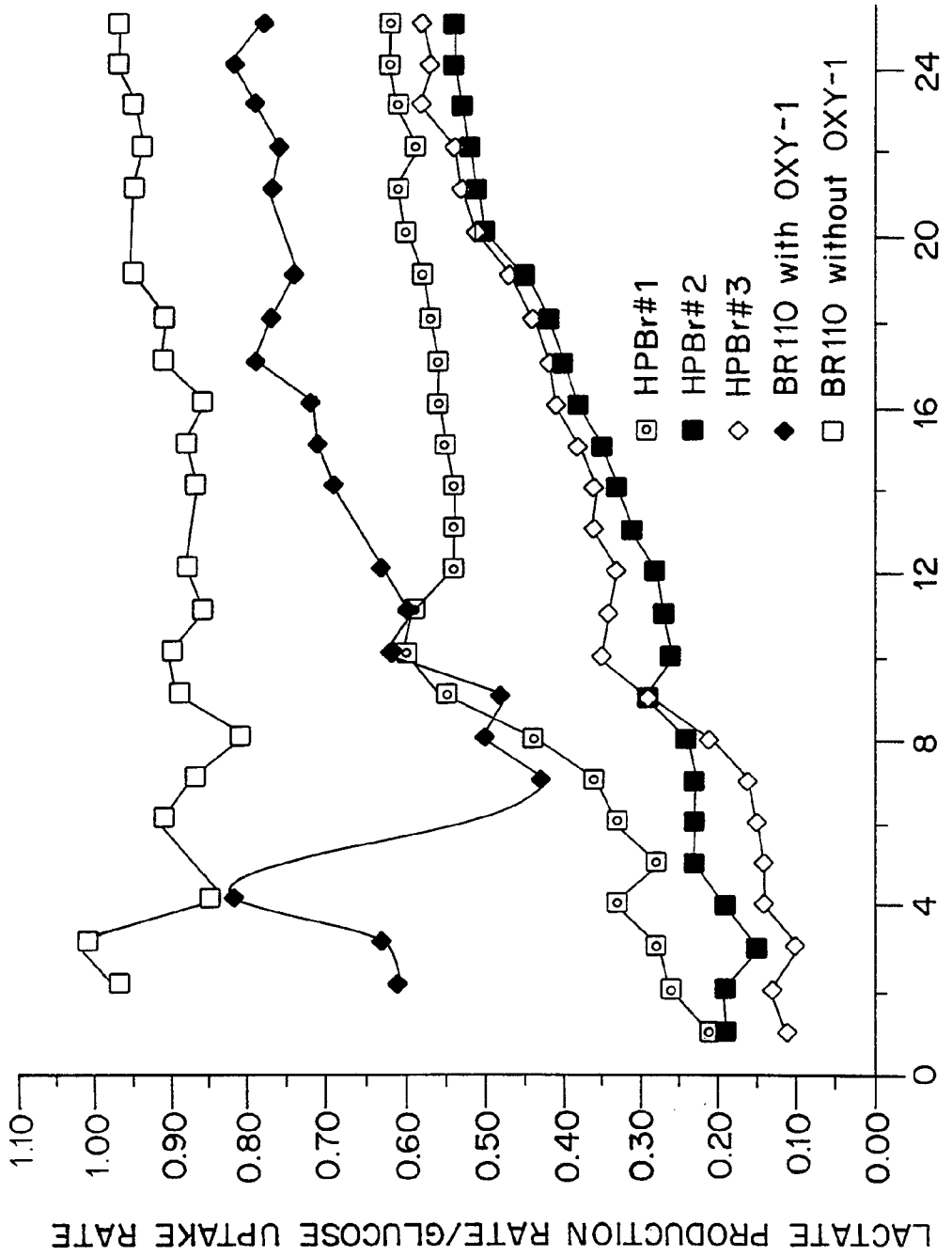
LACTATE PRODUCTION RATE/GLUCOSE UPTAKE RATE
1.10
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0
4
8
12
16
20
24
DAY
HPBr#1
HPBr#2
HPBr#3
BR110 with OXY-1
BR110 without OXY-1

FIG. 16

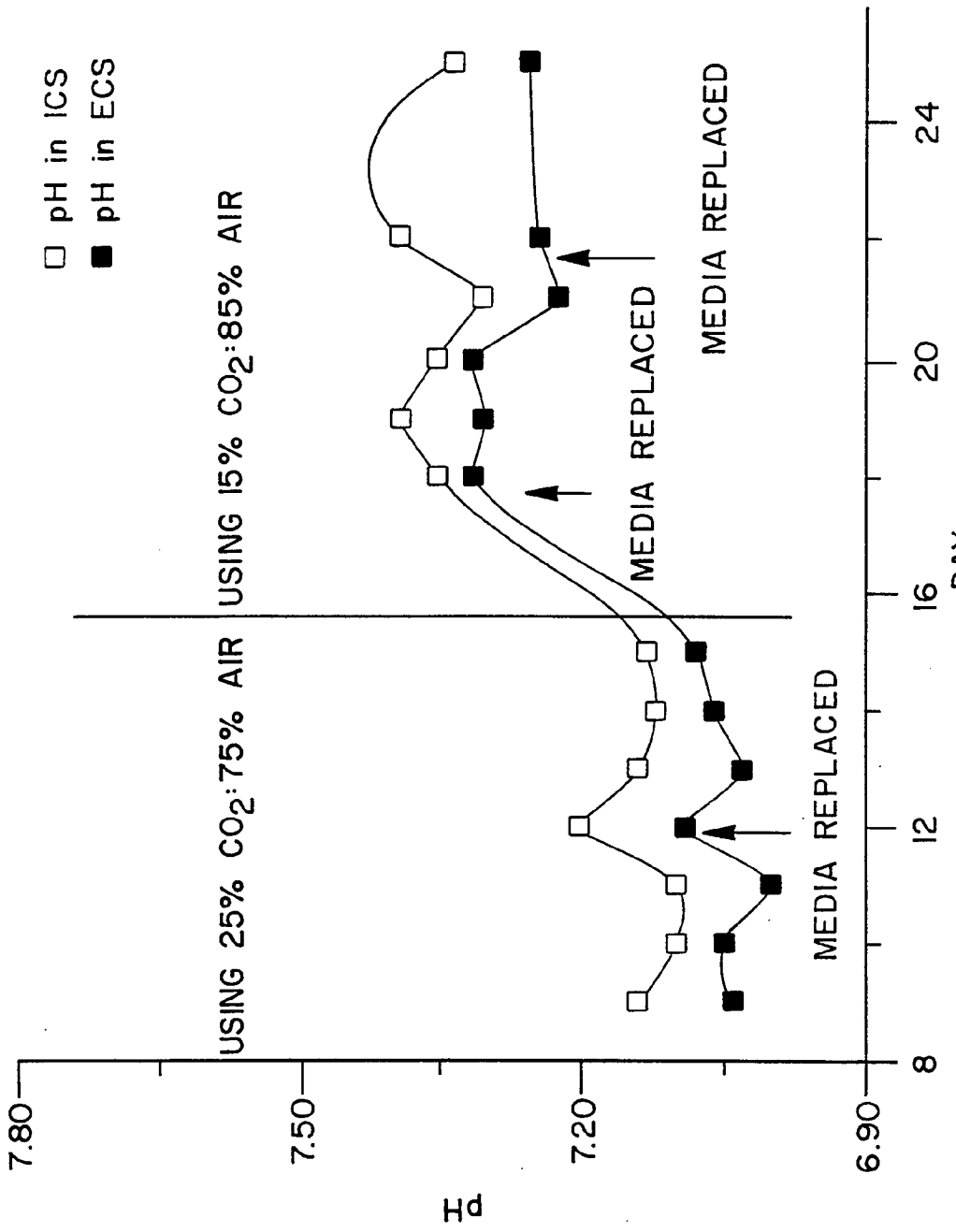
□ pH in ICS
■ pH in ECS
USING 15% CO_2:85% AIR
USING 25% CO_2:75% AIR
MEDIA REPLACED
MEDIA REPLACED
MEDIA REPLACED
7.80
7.50
7.20
6.90
pH
8
12
16
20
24
DAY

FIG. 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/02140

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 5/06; C12M 3/06
US CL :435/240.23, 284
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.23, 240.241, 240.242, 283-286, 311; 422/48; 210/321.64, 321.78, 321.79, 321.80

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,220,725 (KNAZEK ET AL) 02 September 1980, see entire document.	1-17
Y	US, A, 4,720,462 (ROSENSON) 19 January 1988, see entire document.	1-17
Y	WO, A, 86/06094 (BERSILLON) 23 October 1986, see entire document.	1-17
Y	US, A, 5,278,063 (HUBBELL ET AL) 11 January 1994, see entire document.	7
Y	US, A, 4,061,736 (MORRIS ET AL) 06 December 1977, see column 15, lines 11-20.	9,10
Y	WO, A, 92/11355 (EMERSON ET AL) 09 July 1992, see entire document.	13,17

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 JULY 1994	09 AUG 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM H. BEISNER Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*